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Genetic and phenotypic effects on reproductive outcomes for captively-reared coho salmon, *Oncorhynchus kisutch*

J. Louise Conrad [a,*], Elizabeth A. Gilbert-Horvath [b], John Carlos Garza [b]

[a] *Pacific States Marine Fisheries Commission, Warm Springs Hatchery, 3246 Skaggs Spring Road, Geyserville, CA, 95441, USA*
[b] *Fisheries Ecology Division, Southwest Fisheries Science Center, National Marine Fisheries Service and University of California, Santa Cruz, 110 Shaffer Road, Santa Cruz, CA, 95060, USA*

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ABSTRACT

Captive breeding programs are a common approach to preventing extinction and rehabilitating endangered stocks of Pacific salmonids. To minimize inbreeding in these typically small populations, genetic data from microsatellite loci have been used to estimate relatedness and choose spawning pairs. Phenotypic attributes (e.g., body size), that result at least in part from environmental conditions during rearing likely affect reproductive outcome as well. However, the combined effects of individual phenotype and genetic broodstock management have not been previously evaluated. This study assessed the influence of genetic background (source of original broodstock collection, and heterozygosity of both male and female parents), the molecular genetic-derived relatedness coefficient of mated pairs, and phenotypic attributes of female parents (body size, ovulation rate) on reproductive outcome for three brood years of endangered coho salmon, *Oncorhynchus kisutch*, from the Russian River, California. Over 1200 full-sibling family groups were created in total, whose survival was tracked individually from fertilization through the swim-up fry stage. Strong maternal influence on reproductive outcome was found, as increased female body mass resulted in lower progeny survival rates, and higher ovulation rates predicted improved progeny survival. Male and female heterozygosity was generally positively related to embryo survival, but this effect was not consistently observed across brood years or early life stages. The relatedness coefficient between mated pairs had a significant and negative effect on progeny survival, particularly after hatching, even though the most inbred matings were prevented. Thus, use of genetic broodstock management to guide selection of salmon breeding pairs increases offspring survival, in addition to reducing inbreeding.

1. Introduction

Captive breeding programs are an increasingly common approach for preventing extinction of species and populations (Hilton-Taylor et al., 2009; Seddon et al., 2007), including fishes (Fraser, 2008; George et al., 2009). These programs typically involve wild-caught animals that are bred in captivity for the purpose of artificial propagation and/or development of a captive broodstock to maintain genetic resources. They are usually last-resort efforts to prevent extinction when population sizes and natural reproduction rates are too low for recovery to occur without intervention (George et al., 2009). In addition to providing refugia for imperiled populations, captive breeding programs are often undertaken to re-introduce artificially propagated animals into habitat where they have been extirpated. Salmonid species have been the focus of many captive breeding programs because (1) their socioeconomic importance makes their recovery a conservation priority, (2) existing hatchery facilities can be adapted for raising salmonids to reproductive maturity, and (3) their high fecundity and relatively short generation time (2–7 years) make them appropriate candidates for such programs (Flagg and Nash, 1999; Pollard and Flagg, 2004).

Despite the widespread application of captive breeding, the approach is fraught with challenges (Snyder et al., 1996). Two principal difficulties are: (1) maintaining genetic diversity in a small relict population that is likely to have already experienced substantial loss of diversity (George et al., 2009; Kalinowski et al., 2012), and (2) achieving broodstock survival and successful reproduction (McLean et al., 2008; Pollard and Flagg, 2004; Venditti et al., 2013). Maintenance of genetic diversity is imperative for individuals re-introduced into natural habitat to have the capacity to respond to environmental variability, avoid harmful introgression with natural populations (Utter and Epifanio, 2002), and to sustain reproductive fitness into ensuing generations (Araki et al., 2007, 2008, 2009). Many reproductive traits are heritable in salmonids (Carlson and Seamons, 2008), and often reveal substantial maternal influence (Beacham and Murray, 1985; Berg et al., 2001). Paternal effects generally have less influence on offspring survival than maternal effects (Green, 2008; Nagler et al., 2000), but in some cases the male parent may still have significant

* Corresponding author at: Department of Water Resources, 3500 Industrial Boulevard, West Sacramento, CA, 95691, USA. Tel.: +1 1 530 304 0327; fax: +1 1 530 756 4770.
E-mail address: louiseconrad9@gmail.com (J.L. Conrad).

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influence on reproductive outcome (Houde et al., 2011). However, for most hatchery programs, it is difficult to tease apart maternal and paternal influences on embryo survival because eggs from multiple females and/or the same female spawned with different spawn partners are commonly pooled together at very early life stages (Campton, 2004; Neff et al., 2011). In captive breeding programs where successful broodstock reproduction is often a challenge, a critical first line of investigation is to understand whether variability in reproductive outcomes is influenced more by male or female parents.

Given the heritability of fitness-related traits, maintenance of genetic diversity in captive broodstock programs may be critical for the reproductive success of fish spawning in captivity. Minimization of inbreeding is a key aspect of maintaining genetic diversity. In the absence of pedigree information (which is almost never available for natural-origin fish), inbreeding can be reduced by estimating the degree of relatedness between breeders from molecular genetic data (Blouin, 2003). Several estimators of pairwise relatedness coefficients have been developed (Lynch and Ritland, 1999; Queller and Goodnight, 1989; Ritland, 1996), and are now used in captive broodstock programs to rank favorability of potential breeding partners (e.g., Kozfkay et al., 2008).

Despite considerable progress in maintaining genetic diversity of captive populations (Araki and Schmid, 2010; Fisch et al., 2013; Kalinowski et al., 2012), there is still substantial uncertainty about the effects of genetic diversity on reproductive performance (Neff et al., 2011). Because most pairwise estimators of relatedness are based on allelic variation at loci that are not believed to be directly affected by selection, any correlative relationship between alleles at these loci and reproductive performance or other fitness-related traits is not known (Fraser, 2008; Neff et al., 2011). Furthermore, for captive broodstock programs that are entirely reliant on captive-bred broodstock, any positive outcome of genetic management for reproductive performance should increase the viability of the captive population, as well as of the released progeny.

In addition to genetic variation, phenotypic variation also contributes to variability in reproductive success. In salmonids, maternal effects are common and female reproductive traits (e.g. spawn timing, body size at spawning, egg size) likely contribute to variability in offspring survival (Quinn, 2005). While many of these characteristics are heritable, environmental factors also contribute to the spawning phenotype. In captivity, food resources and the rearing environment are very different from conditions in the wild and different genotypes are likely to respond uniquely to the captive environment, potentially resulting in an altered distribution of fitness-related phenotypes. Natural-origin salmonids spawned in captivity typically exhibit wide variation in offspring survival rates (Pollard and Flagg, 2004; Venditti et al., 2013), resulting in unequal family sizes, which in turn contributes to reduced effective population size and genetic diversity (Borrell et al., 2011).

Here, we utilize data from a captive population of coho salmon, *Oncorhynchus kisutch* to investigate maternal effects and the genetic and phenotypic factors influencing reproductive outcomes. This captive broodstock population is part of a reintroduction effort on the central coast of California, U.S.A., where the coho salmon is listed as endangered under the U.S. and California Endangered Species acts and where many populations have been extirpated (Brown et al., 1994). Like many nascent captive broodstock programs, the initial years of breeding were characterized by wide variation in reproductive success. As the population was subject to intensive genetic management and detailed monitoring of offspring survival from individual breeding pairs, it was well-suited for investigation of key sources of variability in reproductive success and the effects of genetic management on reproduction. Specifically, we used genetic and phenotypic data from three different brood years to address the following questions: (1) Is the variation in offspring survival derived principally from male or female parents?; (2) What is the relative influence of genetic (i.e. parental heterozygosity, broodstock source) and phenotypic factors (female size, ovulation rate) on offspring survival?; and (3) Does genetic management to minimize inbreeding influence offspring survival?

2. Materials and methods

2.1. Broodstock program

The Russian River Coho Salmon Captive Broodstock Program (RRCSCBP) was initiated in 2001 in order to prevent the imminent extirpation of coho salmon from the Russian River watershed and to reintroduce fish into suitable habitat within the basin. The founding broodstock were collected as natural origin young-of-the-year during 2001–2003 from Green Valley Creek (GVC) and Dutch Bill Creek (DBC), two tributaries to the Russian River. Fish were transported to the Don Clausen/Warm Springs Hatchery (WSH), on the Dry Creek (DC) tributary of the Russian River (38.71828 N, −122.99977 W). Upon maturation at three years of age (or occasionally two years for males), these fish were spawned and a portion of the offspring held at the hatchery to be used as broodstock in case insufficient numbers of naturally produced juveniles were available for collection in subsequent years. This situation first occurred in 2004, when natural-origin young-of-the-year were not available in the Russian River basin. As a result, after the first three brood years of broodstock were spawned, only captive-origin broodstock were available for spawning, with the exception of a small number of natural-origin fish collected from GVC and DC.

Russian River coho salmon spawn December–February and brood years (BYs) are named after the year in which the first fish from the stock were born, such that BY02 refers to fish born December 2002–February 2003. We examine reproductive outcomes for BY02, BY03 and BY04, which were spawned in winters 2005–06, 2006–07, and 2007–08, respectively. Husbandry methods for embryo incubation were still being refined during the spawn seasons for the first BYs (BY00 and BY01); thus, the offspring survival data were too inconsistent within BYs to be used for statistical analyses. Nearly all fish were spawned as three-year-olds with a small fraction of males spawning as two year olds and one male spawning as a four year old (Table 1). BY02, collected as juveniles from GVC in 2003, was the last entirely natural-origin brood year ($n = 249$), with BY03 ($n = 191$) and BY04 ($n = 245$) composed almost entirely of captive-origin fish descended from BY00 and BY01 (Table 1).

During the first year of rearing, all fish were raised in indoor flow-through troughs (4.9 m × 0.9 m × 0.6 m). At approximately one year of age, all individuals received Passive Integrated Transponders tags (PIT-tags, Biomark, Inc.) and a small piece of caudal fin tissue was removed for genetic analysis. The following spring, fish were transferred to freshwater, outdoor circular tanks (6.1 m diameter) until maturity. Despite the lack of a saltwater phase in the life cycle, 99% of females and 95% of males that survived to the adult stage successfully completed sexual maturation.

As part of a separate, pilot-scale effort to investigate an alternative broodstock diet, a portion of both BY02 (42 females) and BY03 (43 females) broodstock received a modified diet, beginning in the spring prior to their anticipated spawning the following winter. The standard diet was BioOregon semi-moist pellets (Skretting, Inc., pellet size 6.0 and 8.0 mm) and the alternative diet was composed of 60% blanched, frozen krill (*Euphausia* spp., Krill Canada, Inc.), top-coated with cod liver oil and vitamins, and 40% of the same pellets as in the standard diet. Due to an enhanced feeding response to the inclusion of krill, all broodstock in BY04 were given 60% of their ration in krill starting in the spring prior to their spawning winter. All fish were fed a daily ration equivalent to approximately 2% of their body weight, and food was no longer offered after mid-October, in anticipation of spawning beginning in mid-late December. While the